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Technical Note: Electrochemical and Chemical Complications Resulting from Yeast Extract Addition to Stimulate Microbial Growth

Jason S. Lee[‡,] and Brenda J. Little[*]*

ABSTRACT

Addition of 1 g/L yeast extract (YE) to sterile, aerobic (approximately 21% dissolved oxygen) and deoxygenated (<0.0001% dissolved oxygen) natural seawater fixed the corrosion potential (E_{corr}) of 316L (UNS S31603) stainless steel. YE contains riboflavin and other B vitamins that can act as redox mediators, sorb to surfaces, and chelate metal ions. As demonstrated, YE alters the pH of buffered media, including natural seawater. These same activities are typically attributed to microorganisms and are related to microbiologically influenced corrosion (MIC) mechanisms. Despite the prevalent use of YE to stimulate microbial growth in MIC experiments, the potential impact of YE on the outcome of those experiments has not been examined.

KEY WORDS: 316L, microbiologically influenced corrosion, redox mediators, riboflavin, yeast extract

INTRODUCTION

Yeast extract (YE) is routinely added to microbiological media to encourage microbial growth, including experiments designed to evaluate microbiologically influenced corrosion (MIC), i.e., corrosion caused by the presence and/or activities of microorganisms.[1-4] YE is made by extracting yeast cells (removing the cell walls). Several yeast species and growth media are used for commercial production, including strains of *Saccharomyces cerevisiae* grown on molasses-based media, debittered brewers yeasts (strains of *Saccharomyces cerevisiae* or *Saccharomyces uvarum*), *Kluyveromyces fragilis* fermented on whey, and *Candida utilis* grown on carbohydrates or ethanol. Yeast cells are harvested, washed, resuspended in water, and lysed with enzymes. The resulting extract is filtered and dried into a powder. Because of production variables, the term "yeast extract" does not define a specific chemical composition. Consequently, culture media containing YE are undefined. YE typically contains vitamins (e.g., thiamine, riboflavin, pantothenic acid, pyridoxine, niacin, and cyanocobalamin) in addition to carbohydrates and proteins (Table 1).

While developing a procedure for producing rapid sulfate-reducing bacteria (SRB)-influenced corrosion of Fe-15Cr-10Ni in the laboratory, Webster and Newman[5] reported that YE in their culture medium was a potential "redox poising agent." In redox poising, the availability of electrons is fixed by a predominant redox reaction. Webster and Newman[5] reported problems with electrochemical measurements made in culture media containing YE at concentrations of 1,000, 290, and 57.1 mg/L. They demonstrated that removal of YE from the medium reduced SRB growth rates but also removed the interferences on electrochemical measurements.

Based on the observations of Webster and Newman,[5] Little and Lee,[6] and Lee, et al.,[7] Little, et al.,[8] have communicated potential problems with the addition of YE to electrolytes used for electrochemical evaluations of MIC. However, YE is still used in aerobic and anoxic MIC and MIC inhibition (MICI) experiments at concentrations ranging from 0.5 g/L

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TABLE 1
Specifications for Yeast Extract[9]

Total solids	96.0±2.0%
Total nitrogen	0.5±0.5%
Amino nitrogen	5.2±0.5%
Protein (N×6.25)	65.6±3.0%
Carbohydrates	0.0±3.0%
Vitamin B_1 (thiamine)	10-200 mg/kg
Vitamin B_2 (riboflavin)	60-90 mg/kg
Vitamin B_3 (niacin, nicotinic acid, or nicotinic amide)	900-1,100 mg/kg
Vitamin B_5 (pantothenic acid[(A)])	120-160 mg/kg
Vitamin B_6 (pyridixine)	60-80 mg/kg
Vitamin B_{12} (cyanoconalamin)	5-15 µg/kg

(A) Name according to U.S. National Library of Medicine.[10]

to 5 g/L.[1-4] In addition to electrochemical measurements, mass loss, scanning electron microscopy (SEM), and pit depth profiles have been used to conclude microbial acceleration or inhibition of corrosion in the presence of YE. The following discussion includes a review of the use of YE as a redox mediator in multiple applications and the problems that have been documented. Experiments were designed to demonstrate potential, unintentional abiotic consequences of adding YE to sterile, natural seawater using austenitic stainless steel 316L (UNS S31603)[(1)] under aerobic and deoxygenated conditions. Exposures of 24 h lessened the possibilities of microbial contamination and localized corrosion of corrosion-resistant 316L.

EXPERIMENTAL PROCEDURES

316L cylindrical coupons 1.25 in (3.2 cm) long, 0.188 in (0.48 cm) diameter, and 0.736 in^2 (4.823 cm^2) surface area were acquired from Metal Samples (Part number EL400†). The chemical composition was provided by the manufacturer: Fe, <0.03% C, 16% to 18.5% Cr, 10% to 14% Ni, 2% to 3% Mo, <2% Mn, <1% Si, <0.045% P, <0.03% S.

Natural seawater was collected at the Naval Research Laboratory (NRL) in Key West, FL and shipped overnight to NRL at Stennis Space Center, MS. Seawater was sterilized using a combination of filtration and pasteurization.[11] Seawater was filtered through a 0.22 µm filter, collected in sterile glass bottles, pasteurized in an oven at 70°C for 2 h, and allowed to cool to room temperature (23±1°C) overnight.

Electrochemical measurements were conducted in Model K0047† corrosion cells (Princeton Applied Research). Each cell contained one 316L coupon (working), two graphite rods (counters), and a saturated calomel electrode (SCE, reference) inserted into a saturated potassium chloride-filled Luggin probe. Each 316L coupon was threaded onto a metal rod (providing electrical connection) and suspended vertically in the center of the cell with a glass capillary surrounding the metal rod. A rubber O-ring sealed the metal rod from contact with the interior of the corrosion cell, leaving only the working electrode exposed.

Three h prior to corrosion cell assembly, 316L coupons were hand polished to 600 grit, rinsed with distilled water followed by methanol, and dried with nitrogen gas. Electrochemical cells were assembled with associated working and counter electrodes and Luggin probe in place. A foam stopper was placed in one port hole of each corrosion cell, allowing gas exchange during the experiment. Assembled corrosion cells were sterilized by autoclave for 15 min at 250°C. Reference electrodes were not sterilized but were separated from the interior of the cell by Luggin probe to reduce the probability of microbial contamination.

Sterilized seawater was used for four test solutions: (1) aerobic seawater exposed to laboratory air, (2) deoxygenated seawater bubbled with anoxic gas (Table 2) for 2 h, and (3) and (4) addition of 1 g/L YE (Difco Laboratories) to solutions (1) and (2), respectively. The pH was measured in all treatments using an Accumet† AR50 digital pH meter and adjusted to 8.15 with 0.5 M NaOH, if necessary. Solutions were visually checked regularly for turbidity, an indication of microbial contamination.

Seawater (550 mL) was added to each corrosion cell. The solution level was below the O-ring/working electrode connection and prevented the immersion of unintended crevices from interfering with electrochemical measurements. The area of the working electrode exposed to solution was 4 cm^2. Experiments were conducted at 23±1°C in laboratory air (aerobic) and inside a controlled deoxygenated atmosphere (Table 2) chamber[7] (Coy Laboratory Products). Palladium (Pd) catalyst stacks were placed within the chamber to maintain the atmospheric oxygen level to below 1 part-per-million (ppm) as measured by an oxygen sensor (Model 10 Gas Analyzer†, Coy Laboratory Products). Humidity in the chamber was suppressed with alumina desiccator stacks.

Corrosion potential (E_{corr} [V_{SCE}]) was measured using a Reference 600† potentiostat and a ECM8† Multiplexer (Gamry Instruments). The Luggin probe was placed within 3 mm of the working electrode. Experiments lasted 24 h. Dissolved oxygen in the

TABLE 2
Composition of Exposure Atmospheres in vol%

	N_2	CO_2	H_2	O_2
Laboratory Air[(A)]	78.08	0.038	0.00005	20.95
Deoxygenated	Bal.	0.1	10	<0.0001

(A) Standard dry air.[12]

(1) UNS numbers are listed in *Metals and Alloys in the Unified Numbering System*, published by the Society of Automotive Engineers (SAE International) and cosponsored by ASTM International.
† Trade name.

seawater was not monitored. Solution oxidation-reduction (redox) potential (E_h) was measured at the onset and the conclusion of each experiment with an Orion 9179BN ORP Triode† (Thermo Scientific), standardized with Orion 967961 ORP Standard Solution†. E_h values are reported as mV vs. standard hydrogen electrode (mV_{SHE}). Solution pH was measured at the conclusion of each experiment and 316L surfaces were examined with a Quanta 200 SEM† (FEI Company). Triplicates of each exposure condition were performed and measurements (E_{corr}, E_h, and pH) are reported as average values with associated standard deviations.

RESULTS

Turbidity was not observed over the 24-h exposure period. Localized corrosion (i.e., pitting and crevice corrosion) was not detected on any 316L coupons under SEM examination. Polishing marks were intact on all coupons and microorganisms were not observed on any coupons.

Upon receipt, seawater salinity and pH were $37^{0/00}$ and 8.28, respectively. As a result of the filtration/pasteurization process, seawater pH was 8.15 and salinity was unchanged. pH and E_h of the test solutions at the experimental onset and after 24 h were averaged and standard deviations reported (Table 3). Bubbling seawater with the gas mixture to remove oxygen slightly increased sterile seawater pH to 8.16. Addition of YE caused acidification of the both aerobic and deoxygenated seawaters with a dependence on oxygen content: pH 7.36±0.01 (aerobic) and pH 7.84±0.01 (deoxygenated). As shown in Table 3, pH of all seawaters increased after 24 h to a maximum of pH 8.25 under deoxygenated conditions. Aerobic seawater had the highest E_h (404±5 mV_{SHE}), while deoxygenated seawater with (–627±8 mV_{SHE}) and without (–628±4 mV_{SHE}) YE addition had the lowest. Addition of YE to aerobic seawater substantially decreased E_h to 30±14 mV_{SHE}. After 24 h, deoxygenated seawaters with (–443±16 mV_{SHE}) and without (–470±17 mV_{SHE}) YE addition had statistically similar E_h. Aerobic seawater maintained the highest overall E_h (390±15 mV_{SHE}) but decreased from initial values. In contrast, E_h of aerobic seawater with 1 g/L YE increased to an average of 132±26 mV_{SHE} after 24 h. Aerobic seawater with 1 g/L YE had the largest standard deviations initially and after 24 h.

In aerobic seawater, E_{corr} of 316L increased from –0.02 V_{SCE} to 0.06 V_{SCE} over 24 h (Figure 1). E_{corr} values in deoxygenated seawater were consistently lower by ~0.1 V compared with aerobic measurements (Figure 1). YE addition resulted in an average decrease of 0.08 V in E_{corr} for aerobic seawater (Figure 2). Figure 3 shows the effect of YE addition on E_{corr} of 316L in deoxygenated conditions. E_{corr} was statistically similar with and without YE addition over the 24-h exposure. For exposures with and without YE, E_{corr} was initially ~–0.14 V_{SCE} and increased to near –0.05 V_{SCE} after 24 h. Average E_{corr} values of 316L in aerobic and deoxygenated seawaters with YE addition were nearly identical, with standard deviations overlapping at all times except for the initial points (Figure 4).

DISCUSSION

YE is a source of nutrients, especially nitrogen-containing compounds and carbohydrates that can stimulate the growth of some microorganisms. However, YE does not always have a positive effect on microbial growth. Widdel and Bak,[13] working with gram-negative mesophilic SRB, reported that several SRB were not stimulated by complex nutrients, and for a few, YE appeared to be inhibitory. YE also contains B vitamins, including riboflavin and nicotinnamide (Table 1), known redox mediators. Redox mediators are typically small, soluble molecules capable of accepting and donating electrons.[14] Redox mediators are often added to experimental systems to improve the rate of electron transfer between microorganisms and electrodes.[15] Much of what is known about the redox mediating properties of YE has been established for anaerobic reactions with azo dyes and chloroform and in microbial fuel cells.

Yeast Extract Used in Microbiologically Mediated Anaerobic Reactions with Azo Dyes and Chloroform

Anaerobic reduction of the azo bond depends on the redox potential of the solution and the specific dye.[16] The kinetics of azo dye reduction depends on the dye concentration and on the presence of reducing equivalents, external carbon sources, and redox

TABLE 3

Chemical and Electrochemical Properties of Exposure Seawaters

Atmosphere	Yeast Extract (g/L)	pH (initial)	pH (24 h)	E_h (initial) (mV_{SHE})	E_h (24 h) (mV_{SHE})
Aerobic	0	8.15	8.17±0.01	404±5	390±15
Deoxygenated	0	8.16	8.25±0.04	–628±4	–470±17
Aerobic	1	8.15[(A)]	8.19±0.01	30±14	132±26
Deoxygenated	1	8.15[(A)]	8.25±0.01	–627±8	–443±16

[(A)] pH set by addition of 0.5 M NaOH.

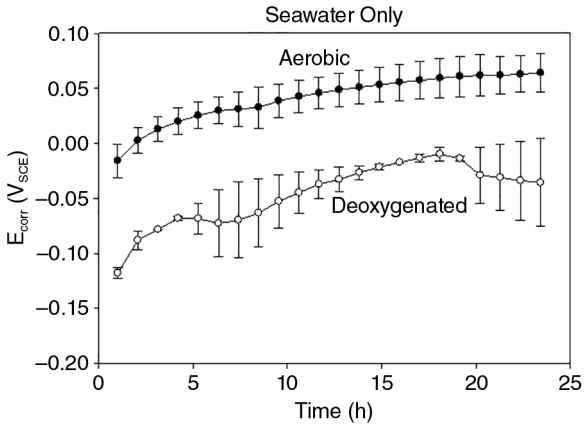

FIGURE 1. *Corrosion potential (E_{corr} [V_{SCE}]) of 316L over 24-h exposure in sterile, natural seawater under aerobic (pH 8.15) and deoxygenated (pH 8.16) conditions. Averages and standard deviations of triplicate exposures are shown.*

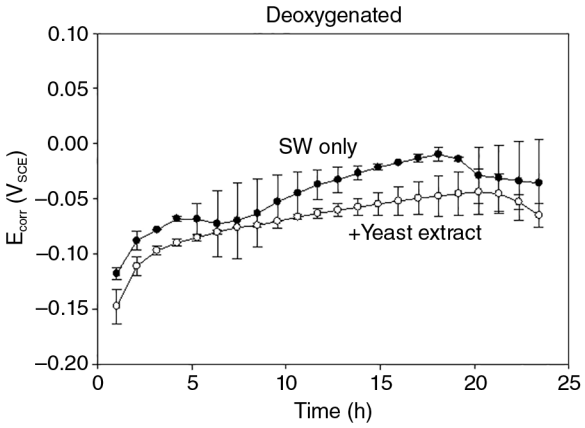

FIGURE 3. *Corrosion potential (E_{corr} [V_{SCE}]) of 316L over 24-h exposure in sterile, natural deoxygenated seawater with 1 g/L yeast extract (+Yeast Extract) and without (SW Only). pH of both seawaters was adjusted to 8.15 prior to experimental onset. Averages and standard deviations of triplicate exposures are shown.*

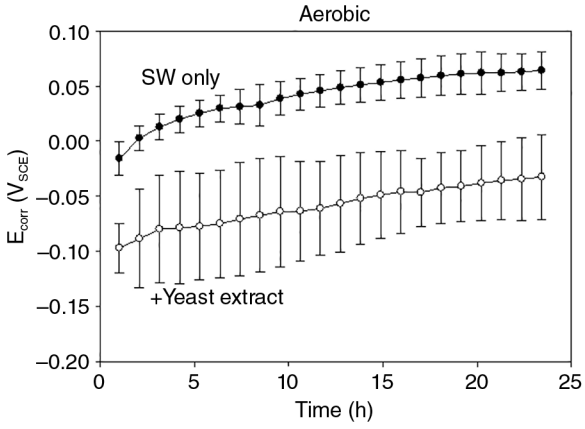

FIGURE 2. *Corrosion potential (E_{corr} [V_{SCE}]) of 316L over 24-h exposure in sterile, natural aerobic seawater with 1 g/L yeast extract (+Yeast Extract) and without (SW Only). Seawater pH was adjusted to 8.15 after yeast extract addition matching the seawater-only value. Averages and standard deviations of triplicate exposures are shown.*

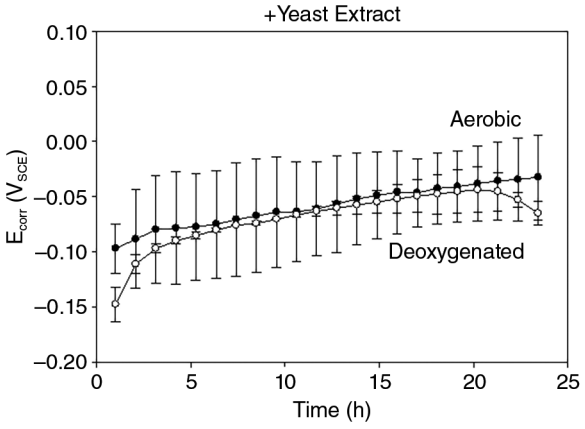

FIGURE 4. *Corrosion potential (E_{corr} [V_{SCE}]) of 316L over 24-h exposure in sterile, natural aerobic and deoxygenated seawaters with 1 g/L yeast extract (+Yeast Extract). As a result of acidification from yeast extract addition, seawater pH was adjusted to 8.15 prior to experimental onset. Averages and standard deviations of triplicate exposures are shown.*

mediators.[17] Correa, et al.,[18] showed that 0.5 g/L YE accelerated the kinetics of anaerobic decolorization of azo dye solutions and attributed the effect to riboflavin. They[18] further concluded that 0.5 g/L YE was simultaneously a source of carbon and redox mediators. Silva, et al.,[19] observed the highest efficiency for color removal in the presence of 0.5 g/L of YE. Avramova, et al.,[20] studying the effect of natural (riboflavin) and synthetic redox mediators on microbial decoloration of acid orange, an azo dye, reported that the influence of the mediator depended not only on the nature and concentration of the mediator, but also on the specific microorganisms used in the process.

Guerrero-Barajas and Field[21] evaluated redox active vitamins (i.e., riboflavin [molar mass 376 g/mol] and cyanocobalamin [molar mass 1,355 g/mol]) as shuttles to enhance the anaerobic biodegradation of chloroform by a consortium of methanogenic microorganisms. They reported only negligible degradation in controls without the redox vitamins and enhanced anaerobic degradation rates at substoichiometric molar ratios as low as 0.1 (37.1 g/L) to 0.01 (13.55 g/L) vitamin:chloroform for riboflavin and cyanocobalamin, respectively. They[21] concluded that riboflavin and cobalamins dramatically increased rates of chloroform biotransformation in very low mol:mol ratios.

Yeast Extract Used as a Redox Mediator in Biofuel Cells

Sayed, et al.,[22] evaluated the impact of YE in an open-air biofuel cell consisting of carbon cloth as an anode and a platinum electrode as the cathode. Anode potentials were measured for different YE

concentrations, i.e., 0.1, 1, 10, and 100 g/L. The anode potential decreased with increasing YE concentration. The authors interpreted the decrease as an indication that YE was responsible for electron transfer. The initial current density was 0.5 μA/cm^2 at 0.1 g/L YE and increased with increasing YE concentration to 7 μA/cm^2 at 100 g/L. For all concentrations, the observed current density decrease with time was attributed to the microbial consumption of reduced components in YE.

Lanthier, et al.,[23] examined current production by *Shewanella oneidensis* in microbial fuel cells with defined media (no YE) and media with 0.5 g/L YE. *Shewanella* species can produce electron shuttles, which permit the microorganisms to reduce insoluble Fe(III) oxides without direct cell-electron acceptor contact.[24] Lanthier, et al.,[23] reported that current production increased in media, with and without YE, to a maximum of 0.2 mA to 0.3 mA with no consistent differences between the two media. However, maximum current was typically reached earlier in the medium containing YE. They[23] reported more planktonic and sessile biomass in the presence of YE. There was no mention of electron shuttles or redox mediators contributed by the YE. Marsili, et al.,[24] detected redox-active molecules within *Shewanella* sp. biofilms, with riboflavin being the dominant component during incubation of >72 h. They further demonstrated sequestration of riboflavin at carbon electrode surfaces and concluded that whenever soluble flavins were present, a percentage was adsorbed to the electrode. They also observed binding of riboflavin to Fe(III) and Mn(IV) oxy(hydr)oxide surfaces.

Yeast Extract Used in Microbiologically Influenced Corrosion Experiments

Zhao, et al.,[4] concluded that Gulf of Mexico and Arabian Gulf seawaters lacked sufficient organic carbon to support the rapid growth of biofilms. Consequently, they added 1 g/L YE, 3.5 g/L sodium lactate, and 200 ppm (mg/L) Fe(II) to the seawaters. At the conclusion of their experiments, coupons were examined with SEM.[4] No pitting was observed in natural seawater. In the augmented seawater samples spiked with SRB, they observed microbial growth and pitting after 1 week.

Using Modified Baar's solution (American Type Culture Collection medium 1249[†] containing 1 g/L YE), Gu and Xu[25] demonstrated that both riboflavin and flavin adenine dinucleotide (FAD) added at a final concentration of 10 ppm increased weight loss and pitting by *Desulfovibrio vulgaris*, without increasing the number of planktonic or sessile cells. They[25] concluded that both riboflavin and FAD were MIC promoters at 10 ppm (0.001 g/L). However, it does not appear that they considered the MIC promoters, including riboflavin, in the YE. One kg of YE contains 60 mg to 90 mg riboflavin (Table 1). One g of YE in 1 L of culture media would result in a concentration of 0.060 mg/L to 0.090 mg/L (ppm) riboflavin. As previously indicated, there are potentially other redox mediators in YE.

Enning, et al.,[26] stated that special SRB can exploit metallic iron as electron source. Their experiments were conducted in an anaerobic artificial seawater medium described by Widdel and Bak.[13] However, it is difficult to determine the final composition of the medium in which the experiments were conducted. Widdel and Bak[13] described a multipurpose medium with 25 stock solutions, some containing nicotinic acid, vitamin B_{12}, and YE or peptone. Without knowing the specific composition, it is impossible to evaluate the concentration of redox mediators that could have been unintentionally added.

Using weight loss as an indication of corrosion, several investigators[27-29] have demonstrated that corrosion acceleration or inhibition of carbon steel by aerobic microorganisms depended on growth media/electrolyte composition. Experiments in all cases were designed to show the impact of medium composition on weight loss resulting from microorganisms and not specifically designed to evaluate the impact of YE. Interestingly, all groups evaluated a medium that contained YE and a medium that was free of YE. However, in all cases other variables were also altered so that the absence/presence of yeast extract cannot be interpreted. For example, in the Javed, et al.,[27] experiments, nutrient broth (2 g/L YE) contained 5 g/L NaCl. Media without YE contained 0.5 g/L NaCl. In all cases, the medium containing YE and the higher concentration of NaCl accelerated corrosion compared to a sterile medium without YE and an order of magnitude less NaCl. Rodin, et al.,[29] made a similar observation with Luria-Bertani medium (5 g/L YE, 10 g/L NaCl) compared with glucose minimal medium with peptone and with no YE and no NaCl, i.e., the uninoculated medium containing YE and NaCl caused dramatically more corrosion than sterile media without either YE or NaCl. Neither group[27,29] related their observations to YE specifically.

Nagiub and Mansfeld[3] compared E_{corr} values for carbon steel (UNS G10100), cartridge brass (UNS C26000), aluminum 2024-T3 (UNS A92024), and 316L stainless steel in artificial seawater (Vataanen nine salts solution [VNSS]). They measured E_{corr} in VNSS with and without growth medium. VNSS with growth medium contained 0.5 g/L YE, 1 g/L peptone, 0.5 g/L starch, and 0.5 g/L glucose. The authors[3] did not specify the oxygen concentration or pH of the media. The E_{corr} values were consistent with a deoxygenated condition. The authors[3] did not observe a consistent electrochemical impact on E_{corr} resulting from addition of growth medium. E_{corr} of aluminum 2024-T3 and cartridge brass decreased, while E_{corr} of carbon steel and stainless steel increased. The authors[3] reported microbial contamination after 3 d in media containing YE.

Laurent, et al.,[30] measured E_{corr} with a gold-palladium alloy (Au 51.2%, Pd 38.6%, In 8.6%, and Ga 1.5%) and a nickel-chromium alloy (Ni 76%, Cr 13%, Mo 3%, Ti 2%, and Be 1.5%) in artificial saliva with YE (5 g/L) and without. The motivation for their study was an evaluation of the corrosion behavior of dental alloys in the presence of bacteria. Their 20-h experiments were conducted at 37°C with rotation (500 rpm) under anaerobic conditions. YE caused a decrease in E_{corr} relative to the sterile artificial saliva without YE (92 mV_{SCE} vs. 56 mV_{SCE} for Au-Pd alloy and –300 mV_{SCE} vs. –360 mV_{SCE} for the Ni-Cr alloy). They attributed their observations to changes in pH (5.5 without YE and 6.5 with YE) and assumed (i.e., not measured) reduction of dissolved oxygen and the presence of organic products in YE. In the absence of other contributing factors, the one unit increase in pH would predictably cause a reduction in E_{corr} of 59 mV.[31]

Abiotic Electrochemical Experiments

Data presented in this paper demonstrated the electrochemical impact of adding 1 g/L YE to either an aerobic or deoxygenated electrolyte (i.e., sterile, natural seawater), using E_{corr} and E_h measurements. E_h is a property of an electrolyte, indicating the oxidizing or reducing tendency of an electrolyte, is electrode-material independent, and is most frequently reported with reference to SHE.[32] For example, E_h typically increases as dissolved oxygen concentration increases to saturation. E_{corr} is the characteristic of a metal in an electrolyte. Byrne, et al.,[33] demonstrated that for high alloy steels, including 316L, in synthetic seawater (no YE), there was a linear relationship between E_{corr} and E_h. However, that relationship does not exist in the presence of YE. Addition of 1 g/L YE to aerobic seawater (E_h = 30 mV_{SHE} [Table 3]) caused a decrease of E_{corr} to –0.12 V_{SCE} (Figure 2), the equivalent of deoxygenation (Figure 1). The effect of the E_{corr} decrease with YE addition was minimal under deoxygenated conditions (Figure 3). Under aerobic conditions, there was little change in E_h over the 24-h exposure. In the absence of YE, deoxygenation resulted in a dramatic decrease in E_h (404 mV_{SHE} to –628 mV_{SHE}, Table 3), indicating a shift from oxidizing to reducing conditions in the seawater. Under aerobic conditions, addition of 1 g/L YE also caused an average decrease in E_h from 404 mV_{SHE} to 30 mV_{SHE}, presumably a result of the chemical oxygen demand of organic constituents in the YE.[30] Under anaerobic conditions, addition of YE did not influence E_h.

Laurent, et al.,[30] reported that addition of 5 g/L YE in anaerobic sterile artificial saliva caused an increase in pH (5.5 to 6.5) and decrease in E_{corr} (92 mV_{SCE} to 56 mV_{SCE} and –300 mV_{SCE} to –360 mV_{SCE}) for precious and non-precious alloys, respectively. This coupled observation is consistent with thermodynamic predictions.[31] Addition of 1 g/L YE to natural seawater (pH 8.15) caused an acidic shift in pH under both aerobic (pH 7.34) and deoxygenated (pH 7.86) conditions. However, all experiments described in this paper were conducted at pH 8.15 to eliminate pH effects. In both aerobic and deoxygenated cases, 1 g/L YE caused a lowering of E_{corr} (Figures 2 and 3). The differences were more significant in the aerobic exposure (Figure 2).

Data included in this paper can be used to conclude that the 1 g/L YE in seawater is a redox poising agent (Figure 4), as postulated by Webster and Newman.[5] Under both aerobic and anaerobic conditions with YE, the E_{corr} of 316L stainless steel was between 0.05 V_{SCE} and –0.10 V_{SCE}. In addition, YE contains redox mediators, sorbs to electrode surfaces,[29-30] and chelates metal ions.[22] Some microorganisms secrete redox mediators, colonize surfaces, and produce extracellular polymers that bind metals. These are the very properties that are essential to understanding the role of microorganisms in MIC experiments.

Cautions in the Use of Yeast Extract

YE is used successfully in anaerobic reactions with azo dyes and the biodegradation of chloroform as both a carbon source and a redox mediator. However, the addition of YE to culture media in biofuel cells is being questioned. Masuda, et al.,[34] concluded that exogenous redox mediators added to media in fuel cells affected the results. They[34] strongly discouraged addition of YE as a source of nutrients in microbial fuel cell media and suggested that previous results obtained in the presence of YE should be re-analyzed. Sayed, et al.,[22] stated that only a few of the functions of YE in biofuel cells are known.

The obvious complexities introduced by addition of YE to microbiological culture media have been acknowledged by those working with microbial fuel cells, but ignored by many MIC investigators. Furthermore, as YE is consumed and microorganisms grow, the influence of YE is expected to change. Specific YE-microorganism reactions make it impossible to generalize about the potential impact of YE on the biotic reactions in MIC experiments.

SUMMARY

In abiotic natural seawater, 1 g/L YE decreased the pH and fixed the E_{corr} of 316L to the same value under both aerobic and deoxygenated conditions. The composition of YE is not specific. Therefore, the chemical and electrochemical consequences of adding YE to stimulate growth of microorganisms in electrochemical experiments may vary with different YE products. Furthermore, because YE constituents are redox mediators, sorb to surfaces, and chelate metal ions, the impact of adding YE to MIC experiments cannot be predicted.

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REFERENCES

1. H. Castaneda, X.D. Benetton, *Corros. Sci.* 50, 4 (2008): p. 1169-1183.
2. X. Dominguez-Benetton, H. Castaneda, "SRB-Biofilm Growth and Influence in Corrosion Monitoring by Electrochemical Impedance Spectroscopy," CORROSION/2005, paper no. 486 (Houston, TX: NACE International, 2005).
3. A. Nagiub, F. Mansfeld, *Mater. Corros.* 52, 11 (2001) p. 817-826.
4. K. Zhao, T. Gu, I. Cruz, A. Kopliku, "Laboratory Investigation of MIC in Hydrotest Seawater," CORROSION 2010, paper no. 406 (Houston, TX: NACE, 2010).
5. B.J. Webster, R.C. Newman, "Producing Rapid Sulfate-Reducing Bacteria (SRB)-Influenced Corrosion in the Laboratory," in *Microbiologically Influenced Corrosion Testing*, eds. J.R. Kearns, B.J. Little, STP 1232 (Philadelphia, PA: ASTM, 1994), p. 28-41.
6. B.J. Little, J.S. Lee, *Microbiologically Influenced Corrosion* (Hoboken, NJ: John Wiley and Sons, Inc., 2007).
7. J.S. Lee, R.I. Ray, B.J. Little, *Corrosion* 66, 1 (2010): p. 15001-1 to 15001-6.
8. B.J. Little, R.I. Ray, J.S. Lee, "Understanding Marine Biocorrosion: Experiments with Artificial and Natural Seawater," in *Understanding Biocorrosion: Fundamentals and Applications*, eds. T. Liengen, D. Feron, R. Basseguy, I.B. Beech, European Federation of Corrosion Publications no. 66 (Amsterdam, Netherlands: Woodhead Publishing, 2014), p. 329-340.
9. Private communication, "Yeast Extract Composition," bioWORLD, http://www.bio-world.com/productinfo/3_43_287_682/1682/Yeast-Extract.html (June 30, 2015).
10. Private communication, "Pantothenic Acid (Vitamin B5)," U.S. National Library of Medicine, http://www.nlm.nih.gov/medlineplus/druginfo/natural/853.html (June 30, 2015).
11. B. Little, S. Gerchakov, L. Udey, *J. Microbiol. Meth.* 7, 4-5 (1987): p. 193-200.
12. Private communication, "Air: Its Composition and Properties," Universal Industry Gases, Inc., http://www.uigi.com/air.html (June 30, 2015).
13. F. Widdel, F. Bak, "Gram-Negative Mesophilic Sulfate-Reducing Bacteria," in *The Procaryotes*, eds. A. Balows, H.G. Truper, M. Dworkin, W. Harder, K.H. Schleifer (New York, NY: Springer, 1992), p. 3352-3378.
14. M. Ammam, J. Fransaer, *Biotechnol. Bioeng.* 109, 7 (2012): p. 1601-1609.
15. D.H. Park, J.G. Zeikus, *Appl. Environ. Microbiol.* 66, 4 (2000): p. 1292-1297.
16. A. Pandey, P. Singh, L. Iyengar, *Int. Biodeter. Biodegr.* 59, 2 (2007): p. 73-84.
17. J.R.M. Willetts, N.J. Ashbolt, *Water Science and Technology* 42, 1-2 (2000): p. 409-415.
18. C.A.R. Correa, S.F. de Aquino, P.C.D. Caldas, S.D. Silva, *Eng. Sanit. Ambient.* 14, 4 (2009): p. 559-568.
19. S.Q. Silva, D.C. Silva, M.C.S. Lanna, B.E.L. Baeta, S.F. Aquino, *Braz. J. Microbiol.* 45, 4 (2014): p. 1153-1160.
20. T. Avramova, L. Stefanova, B. Angelova, S. Mutafov, "Effect of Some Redox-Mediators on the Decolorization of Acid Orange 7 by Resting *Rhodococcus Erythropolis* and *Alcaligenes Faecalis* Cells," in *Current Research Topics in Applied Microbiology and Microbial Biotechnology*, ed. A. Mendez-Vilas (Hackensack, NJ: World Scientific, 2009), p. 581-583.
21. C. Guerrero-Barajas, J.A. Field, *Biotechnol. Bioeng.* 89, 5 (2005): p. 539-550.
22. E.T. Sayed, Y. Saito, T. Tsujiguchi, N. Nakagawa, *J. Biosci. Bioeng.* 114, 5 (2012): p. 521-525.
23. M. Lanthier, K.B. Gregory, D.R. Lovley, *Fems. Microbiol. Lett.* 278, 1 (2008): p. 29-35.
24. E. Marsili, D.B. Baron, I.D. Shikhare, D. Coursolle, J.A. Gralnick, D.R. Bond, *Proceeding of the National Academy of Sciences of the USA* 105, 10 (2008): p. 3969-3973.
25. T. Gu, D. Xu, "Why Are Some Microbes Corrosive and Some Not?" CORROSION 2013, paper no. 2336 (Houston, TX: NACE, 2013).
26. D. Enning, H. Venzlaff, J. Garrelfs, H.T. Dinh, V. Meyer, K. Mayrhofer, A.W. Hassel, M. Stratmann, F. Widdel, *Environ. Microbiol.* 14, 7 (2012): p. 1772-1787.
27. M.A. Javed, P.R. Stoddart, E.A. Palombo, S.L. McArthur, S.A. Wade, *Corros. Sci.* 86 (2014): p. 149-158.
28. S.K. Jigletsova, V.B. Rodin, N.A. Zhirkova, N.V. Alexandrova, V.P. Kholodenko, "Influence of Nutrient Medium Composition on the Direction of Microbiologically Influenced Corrosion," CORROSION/2004, paper no. 575 (Houston, TX: NACE, 2004).
29. V.B. Rodin, S.K. Zhigletsova, N.A. Zhirkova, N.V. Alexandrova, G.V. Shtuchnaja, V.A. Chugunov, "Altering Environmental Composition as a Potential Method for Reversing Microbiologically Influenced Corrosion," CORROSION/2005, paper no. 498 (Houston, TX: NACE, 2005).
30. F. Laurent, B. Grosgogeat, L. Reclaru, F. Dalard, M. Lissac, *Biomaterials* 22, 16 (2001): p. 2273-2282.
31. B.J. Little, F. Mansfeld, "Passivity of Stainless Steels in Natural Seawater," in *Proceedings of the H.H. Uhlig Memorial Symposium*, eds. F. Mansfeld, A. Asphahani, H. Bohni, R.M. Latanision, vol. 94-26 (Pennington, NJ: The Electrochemical Society, 1994), p. 42-55.
32. E. Gileadi, *Electrode Kinetics for Chemists, Chemical Engineers, and Material Scientists* (New York, NY: Wiley-VCH, 1993).
33. G. Byrne, R. Francis, G. Warburton, J. Wilson, "Electrochemical Potential and the Corrosion Resistance of Stainless Steels in SWRO Applications," IDA Conference, paper no. DB09-145 (Topsfield, MA: International Desalination Association World Congress, 2009).
34. M. Masuda, S. Freguia, Y.F. Wang, S. Tsujimura, K. Kano, *Bioelectrochemistry* 78, 2 (2010): p. 173-175.